This is a summary of things I often say when talking about X chromosome inactivation. Perhaps it will be useful. This may not include everything that I will talk about.

**Mammalian X chromosome inactivation - an example of long-noncoding RNA action.**

Why is it that mammalian females go to the trouble of inactivating one of their X's? The reason is that we use differences in the type of sex chromosomes to determine the sex of a cell. Thus, XX = female and XY = male (in mammals). Now the Y chromosome has very few genes (~60) and the X chromosome has many genes (~2000). This causes a problem because, as a general rule, the amount of expression of a gene product is directly proportional to the number of genes that the cell contains. But male and female cells are nearly identical in all of their functions and therefore they "need" to have the same level of gene expression. If, for X-linked genes, the female cell had twice the expression compared to the male cell then it would kill the cell. For the X-linked genes, a mammalian cell needs the amount of gene expression that can be produced by one X chromosome.

To achieve this, the female turns off one of her X chromosomes (aside: this is the underlying reason that Calico and Tricolor cats only occur in females). This turning off of an X chromosome is called DOSAGE COMPENSATION. It causes the dosage of gene expression for X-linked genes to be the same in males and females.

Mary Lyon described X inactivation in 1961. As a result, the inactivation of one X in females is referred to as the Lyon hypothesis. The process is called Lyonization and the inactivated X is called the Barr body. Mammal follow the n-1 rule in which n=# of X chromosomes, n-1=the number of inactivated X's. As a result XXX and XXXX and XXY people are mostly normal (all have 1 functional X).

How does this occur? The X-inactivation center (XIC) is key for this process. It produces two transcripts. One transcript is called Xist and the other transcript is called Tsix. The DNA that encodes these two overlaps. You should imagine it as a single gene with a promoter at each end. The Xist promoter points to the right and the Tsix promoter points to the left. The names of these transcripts should prove useful in remembering this idea.

Before the female cell differentiates (changes from a stem cell to become something else) both X chromosomes express Tsix but do not express Xist. Expression of Tsix keeps Xist off. It appears that a protein that needs to bind the Xist promoter cannot do it when transcription running through it in the "wrong" direction.

What happens next is that one X stops expressing Tsix and Xist is expressed. The 17000 n Xist RNA binds to the Xist gene starts coating the X that expressed it but does not coat the X that did not express it. That is, the coating occurs in cis. The initial coating may start at Xist but it soon spreads to the entire chromosome. This coated X is the one that will be inactivated. The only places that it does not coat are the small number of pseudoautosomal regions that are still active in the Barr Body.

How is it that only one chromosome expresses Xist? The original hypothesis for how this might occur was first described in Rastan's Blocking Factor Model. THIS HYPOTHESIS IS NOT COMPLETELY CORRECT, BUT IS IT USEFUL FOR UNDERSTANDING THE PRINCIPLE. In this model, there is a protein that is available in limiting
quantity. For the sake of discussion, let's pretend that there is only one of these molecules. It is floating around the cell and can bind only one Xist gene. Whichever X it binds is the one will remain the active X (Xa). Xa will never express Xist. Rastan's model is a negative one.

OK, so Rastan's model is not quite correct but it got us thinking in the right direction. The way that it is now described is similar except that a protein(s) does not block Xist but stimulates Tsix expression. The way that we now think that this happens is that there is a protein(s) that binds the near the promoter region of Tsix. Before X inactivation both chromosomes have a small amount of it. This causes both of them to express a small amount of Tsix. Then the Xic region of the X's pair up and the protein will transfer from one X to another. Why does it transfer? Probably because it is a protein(s) that can multimerize with itself. If it encounters more of itself it must multimerize. When the Xic regions come together, the protein(s) multimerize. If one X gets a little bit more of the protein then it will have an advantage because it will then be even more likely to bind more of the protein. This causes a stochastic transfer of the protein from one X to the other (Much of this paragraph to this point is my interpretation of the literature). Once an X has most of the protein then Tsix expression goes way down and Xist expression goes way up for

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**Rastan’s Blocking Factor model**

**Inactivation is a multi-step process**

![Diagram](image-url)
this chromosome. This will be the Xa. The X that looses all of the protein will not express Tsix. The only thing that was keeping Xist off was Tsix expression and so the level of Xist jumps way up. This will be the Xi chromosome. The Figure that shows this is Figure 2 from the Froberg et al. (2013) reference.

How does Xist expression cause X inactivation? The Xist RNAs that are expressed will bind all across the X chromosome in cis. Proteins (& maybe other RNAs) then bind the Xist RNA. These proteins are enzymes and they modify the histones around them. Let's call the modifications "Marks". Imagine that there are histone marks that promote euchromatin (chromatin that is open enough to use) and marks that promote heterochromatin (chromatin that is shut down). Some enzymes that bind Xist remove a euchromatic mark and others add a heterochromatic mark. These marks can then recruit (bind) other enzymes that add a new heterochromatic mark or remove an additional euchromatic mark. In the figure below, we see a timeline that begins with Xist, then H3K4 is demethylated (loss of a euchromatic mark), H3K9 is deacetylated (loss of a euchromatic mark), then H3K27 is methylated (addition of a heterochromatic mark), etc etc. General Histone deacetylation and H3K9 methylation are also pro-heterochromatic. Macro H2A is a replacement for histone H2A that is pro-heterochromatic. DNA methylation is CpG methylation. For many of these steps we are looking at a chain reaction in which one change helps to precipitated the next change. In addition, some later changes can then cause the occurrence of previous changes (think: I cause you, you cause me).

OK, so this is nice and complicated. But wait it gets worse! (or maybe it gets better? well, definitely more interesting.) When does all of this happen? Let's look at what is known from mice. What happens is that during the development of the zygote to the early embryo, an X is inactivated once, and then it is reactivated, and then an X is inactivated again, and then for some cells it is reactivated. Let's try to sort this out.

In the Zygote, we have two Xa's. At about the 4 cell stage we see that the PATERNAL X is inactivated (Xist mechanism). Xp is the name of the paternal X and Xpi is the name of the inactivated paternal X. Development continues Xpi persists in extraembryonic tissues. Then Xp is reactivated in cells destined to become the embryo. NOW we get the coin flipping event in which Xp or Xm is selected for inactivation to become Xi. In the early embryonic tissue, some of the cells will become the germline. In these germline cells, both X's are must be usable and so the Xi in the germline cells must be reactivated!!!

The Xist and Tsix RNAs are now generically called Long NonCoding RNAs because they do not encode a protein. They are abbreviated Lnc RNA.

Xist and Tsix are not the only two Lnc RNAs in existence. Xist and Tsix are just extreme examples. This type of mechanism is used to turn down, or turn up smaller regions of chromosomes. It appears to be just one more way that the cell regu-
lates gene expression. In some cases, the regulation is in cis (like Xist) and in other cases it is in trans.

Other non-coding RNAs (not all Long) are those involved in splicing, RNA editing, RNA modification, RNA interference, miRNA, and telomere synthesis and maintenance.

The references for these two pages are given in the lecture notes.